REMARKS/ARGUMENTS

Claims 1-14 and 21-24 are active. Claim 1 and its dependents have been revised to use the singular term "cell". Claim 1, which employs open claim language, is to be construed as encompassing a method involving one or more cell(s). In some claims the definite article "the" has been replaced by the indefinite article "a" for clarity. Claim 24, which was indicated as allowable, has been placed in independent form. No new matter is believed to have been added. Favorable consideration of this Amendment and allowance of this application are now respectfully requested.

Restriction/Election

The Applicants previously elected with traverse **Group I** claims 1-4, directed to producing a heterologous RNA of interest. The requirement has been made FINAL. The Applicants respectfully request that the claims of the nonelected group(s), or which are directed to other withdrawn subject matter, which depend from or otherwise include all the limitations of an allowed elected claim, be rejoined upon an indication of allowability for the elected claim, see MPEP 821.04.

Aspects of the Invention

As discussed already in the response to the first and the second Office Actions, the claimed method allows to specifically produce a heterologous RNA of interest (any specific RNA molecule which is not encoded by the yeast mitochondrial genome) *in vivo*, in the mitochondria of a synthetic rho$^-$ yeast transformed with a mitochondrial transcription vector comprising a DNA encoding said heterologous RNA of interest under the control of mitochondrial transcription regulatory element(s) that are functional in yeast mitochondria

and a mitochondrial transformation reporter gene (*COX2* gene for example) or a fragment thereof.

The mitochondrial transcription vector (for example, a plasmid) comprises a synthetic mitochondrial transcription unit in which the isolated DNA encoding said heterologous RNA of interest is inserted between a promoter and a terminator that are functional in yeast mitochondria. For example, the DNA encoding said heterologous RNA is flanked by signal sequences for expression of a mitochondrial gene (page 9, lines 10-17; example, page 20, lines 11-17 and figure 3).

As mentioned in the specification (page 4, lines 20-25; page 7, line 31 to page 8, line 17) and demonstrated in the example (page 26, line 28 to page 27, line 4 and figure 4), the claimed method allows to specifically produce said heterologous RNA in large amounts, for a low cost, and in a form which is stable and can be readily isolated. The purification of the RNA of interest is very easy since **the only RNAs produced in the mitochondria of the synthetic rho$^-$ yeast are the RNA of interest alone** (when the reporter gene is not transcribed) **or with the RNA of the reporter gene** (when the reporter gene is not transcribed; page 9, line 27 to page 10, line 9 of the specification). In addition, the DNA used for the transformation of said synthetic rho$^-$ yeast is transcribed to RNA, but this RNA is not translated to protein (page 12, lines 17-21 of the specification).

Definitions

A rho$^+$ or ρ^+cell is a cell (yeast or mammalian) comprising an intact and functional mitochondrial DNA or a mitochondrial DNA carrying local alteration in its sequence which inactivate the respiratory function of mitochondria (rho$^+$ mit$^-$ yeast; page 5, lines 31-36 of the present application).

As explained already in the response to the previous Office Actions, a "mitochondrial transformant" is a yeast whose mitochondria have been transformed with an isolated DNA (usually a plasmid) comprising a foreign DNA of interest and a marker for the yeast mitochondrial genome (mitochondrial transformation reporter gene), using the biolistic bombardment method described in Bonnefoy, *et al.*, Meth. Enzymol., 2001, 350, 97-111 (Bonnefoy 2001) and Bonnefoy, *et al.*, Mol. Gen. Genet., 2000, 262, 1036-1046 (Bonnefoy 2000).

When the yeast which is transformed using the biolistic bombardment method is a yeast lacking mitochondrial DNA (rho^0 or ρ^0 strain), the resulting "mitochondrial transformant" is a synthetic rho$^-$ yeast.

By contrast, a "mitochondrial recombinant" is a recombinant rho$^+$ yeast having inserted foreign DNA into the mitochondrial DNA (mtDNA) by homologous recombination. The mitochondrial recombinant may be obtained by direct integration of the foreign DNA into the mtDNA of a mutant rho$^+$ yeast (see Bonnefoy 2001, page 109-111, "*Transformation of rho$^+$ cells with plasmids or linear DNA fragments*"; Bonnefoy 2000, page 1039, 2nd column, "*Mitochondria transformation*", lines 20-30). However, it is usually obtained by a two step method (see Bonnefoy 2001, pages 105-109 "*Strategies for gene replacement in Saccharomyces cerevisiae mtDNA*"; Bonnefoy 2000, page 1039, 2nd column, "*Mitochondria transformation*", lines 1-20).

This fundamental difference between a mitochondrial transformant and a mitochondrial recombinant of yeast is explained in the definitions pages 5-7 of the specification and illustrated in figure 2 of the present Patent Application.

Rejection--35 U.S.C. § 102

Claims 1, 2, 5, 8-10, 21, 22 and 23 were rejected under 35 U.S.C. § 102(b) as being anticipated by Bonnefoy *et al.*, Mol. Gen. Genet., 2000, 262, 1036-1046 (Bonnefoy, 2000).

This rejection cannot be sustained because the cited prior art does not disclose all the elements of the claimed method for producing a heterologous RNA of interest.

Bonnefoy 2000 pertains to the examination of normal and aberrant translation initiation (*i.e.*, protein production) in *Saccharomyces cerevisiae* mitochondria (mt). Mitochondrial recombinants of *S. cerevisiae* (recombinant rho+) having inserted a chimeric mt-reporter gene (*cox2(1-91)::ARG8^{m}*) into mtDNA (Abstract, lines 1-5, page 1036) were constructed using the two-step biolistic transformation method mentioned above (page 1039, 2nd column, 1st and 2nd paragraphs).

The Examiner argues (page 3 of the instant Office Action) that "*Yeast mitochondria are isolated and RNA is extracted and purified*". However, this is not true.

Bonnefoy 2000 teaches that mitochondrial translation initiation was examined by measuring the expression of a soluble reporter enzyme, Arg8p, in total cellular protein from the recombinant rho+ yeasts (page 1037, 1st column, last paragraph; page 1040, first column , 2nd paragraph; figures 1B and 3; Discussion, page 1042 to page 1043, 1st column, line 4). In addition, steady-state level of the chimeric *cox2(1-91)::ARG8^{m}* mRNA were measured in total cellular RNA from the recombinant rho+ yeasts (page 1039, 2nd column, last paragraph to page 1040, first column, first paragraph "*Analysis of cellular RNA and protein*" and figure 4). Therefore, Bonnefoy 2000 neither discloses nor suggests that yeast mitochondria were isolated and RNA is extracted and purified from the mitochondria.

The Examiner argues on page 3 of the OA that:

> *Absent evidence to the contrary, the DNA encoding the RNA was under control of promoter and terminator that are functional in yeast mitochondria, since the RNA was successfully produced in yeast mitochondria.*

However, Bonnefoy 2000 does not teach producing RNA. Bonnefoy 2000 teaches producing a reporter protein (Arg8p).

In addition, Bonnefoy 2000 teaches that the *ARG8*^{m} can be phenotypically expressed as a reporter for mitochondrial gene expression when inserted into yeast mtDNA (page 1037, 1st column, beginning of 3rd paragraph; page 1040, first column, middle of the last paragraph). Therefore, Bonnefoy 2000 teaches a reporter plasmid comprising a reporter gene for mitochondrial gene expression (*cox2::ARG8*^{m}) which is expressed in recombinant rho+ yeasts to produce a soluble reporter enzyme (Arg8p).

Bonnefoy 2000 neither discloses nor suggests that the *ARG8 r*eporter gene is expressed in the mitochondria of synthetic rho^{-} yeasts. Therefore, Bonnefoy 2000 does not disclose or suggest the step of transforming the mitochondria of yeast cells lacking mitochondrial DNA (rho^{0} cells) with a mitochondrial transcription vector comprising a gene which is expressed in synthetic rho^{-} yeasts to produce a heterologous RNA of interest (*i.e.*, step 1 of the claimed method).

Furthermore, Bonnefoy 2000 does not disclose or suggest:

- the step of culturing the synthetic rho^{-} yeasts (step 3 of the claimed method);
- the step of isolating the mitochondria from said synthetic rho^{-} yeasts (step 4 of the claimed method), and
- the step of extracting and purifying the heterologous RNA of interest from said mitochondria (step 5 of the claimed method).

Therefore, Bonnefoy 2000 does not disclose the claimed method of making a heterologous RNA of interest.

In view of the significant differences between the subject matter taught by Bonnefoy and the invention, this rejection cannot be sustained.

Rejections—Obviousness on Pages 4-11 of OA

The obviousness rejections on pages 4-11 of the OA are each based in part on Koob, et al., U.S. 2009/0098653. However, this reference is not prior art in view of the attached certified English translation of the present application's priority document, FR 0309024.

U.S. 2009/0098653 published on April 16, 2009 (*i.e.* after the filing date of the present Application [July 22, 2004]) and claims the benefit of provisional Application No.60/562,642 filed April 15, 2004.

The present application claims the benefit of FR 0309024 filed July 23, 2003, which was before the April 15, 2004 filing date of provisional Application No. 60/562,642. Moreover, claim 1 of FR 0309024 demonstrates the descriptive support for claim 1 in the present application by describing five steps of the claimed method as well as the transformation of rho^0 yeast cells (see step [1]) which are cells lacking mitochondrial DNA, see also the paragraph bridging pp. 2-3 of the priority document. Support for rho^0 cells of claim 2 is also found in these sections of the priority document. Descriptive support for the modified strains of claim 3, such as the ΔSUV3 is found on page 9, lines 24-35 of the English translation of FR 0309024. Page 9 of the priority document does not name the specific strain ΔDSS1 strain required by claim 3. However, as indicated on page 11, lines 27-28, the ΔDSS1 strain is also referenced to Dziembowski, et al., J. Biol. Chem. 278: 1603. Claims 5-14 and 21-24 also find descriptive support in FR 0309024. Therefore, U.S. 2009/0098653 which claims the benefit of provisional Application No.60/562,642 filed after FR 0309024 is not prior art for claims 1, 2, 3 in part (ΔSUV3 strain), 5-14 and 21-24, though possibly applicable against claim 3 in part (ΔDSS1 strain) and claim 4. Consequently, as explained in more detail below, these rejections cannot be sustained.

Rejection—35 U.S.C. §103

Claims 1, 2, 5, 7, 8, 11 and 21-23 were rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2001, in view of Koob, et al., U.S. 2009/0098653. Koob, et al., U.S. 2009/0098653 is not prior art for the subject matter of claims 1, 2, 5, 7, 8, 11 and 21-23 for the reasons explained above. With regard the solitary teachings of Bonnefoy, 2001, page 2 of the last Office Action indicates that the Applicants arguments distinguishing this reference were persuasive. Consequently, this rejection cannot be sustained.

Rejection—35 U.S.C. §103

Claim 3 was rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2001 and U.S. 2009/0098653 as applied to claims 1, 2, 5, 7, 8, 11 and 21-23, and further in view of Dziembowski *et al.*, JBC, 2003, 278, 1603-1611. This rejection is not sustainable over the combination of Bonnefoy 2001 and U.S. 2009/0098653 because the cited prior art does not disclose all the elements of the method as claimed in claim 1, suggest the combination of elements of the claimed method, or provide a reasonable expectation of success for producing a heterologous RNA of interest using this method.

Furthermore, Dziembowski does not suggest the elements missing from the two primary references, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA transformed with a mitochondrial transcription vector that is functional in yeast mitochondria as discussed already in the response to the previous Office Action.

U.S. 2009/0098653 pertains to transgenomic mitochondria (mitochondria containing a complete and viable exogenous mitochondrial genome) and transmitochondrial cells and organisms containing a complete and viable exogenous mitochondrial genome in the mitochondria (paragraphs [0002], [0004], [0019], [0025], [0033]).

Example 14 discloses the making of transmitochondrial yeasts using the biolistic bombardment method described in Bonnefoy 2001 (see page 10, 1st column, end of paragraph

([0062]). The DNA that is targeted to the yeast mitochondria contains the full unaltered mouse mitochondrial DNA genome (title of example 14, page 9; paragraphs [0065], [0066], [0067]; figure 5) and the complete *COX2* gene from yeast mtDNA. The transmitochondrial yeast contains an entire mouse mitochondrial genome in its mitochondria rather than its own yeast mitochondrial genome ([0019]).

Example 14 teaches that the full mouse mitochondrial genome replicates in the yeast mitochondria independently of the transcription from the mouse mitochondrial promoters (title of example 14, page 9 and paragraph [0067]).

Example 15 (pages 10-11) and paragraphs [0019] and [0033] teach that there is a species-specific control of mitochondrial transcription. Total RNAs from transmitochondrial yeast cell cultures were collected (paragraph [0071]) and the level of yeast *COX2* and mouse *HSP* transcripts was measured by primer extension (paragraph [0075]). The *COX2* reporter gene but not the mouse mitochondrial genome is transcribed in the mitochondria of the transmitochondrial yeast because mouse mitochondrial promoters are not functional in yeast (paragraph [0075]). Therefore, U.S. 2009/0098653 discloses the making of a transmitochondrial rho+ yeast.

U.S. 2009/0098653 does not disclose or suggest the making of a synthetic rho$^-$ yeast according to steps 1 and 2 of the claimed method. The plasmid taught in U.S. 2009/0098653 is not a mitochondrial transcription vector according to the present invention (as defined above) for the following reasons:

- the plasmid does not contain a DNA encoding said heterologous RNA of interest under the control of mitochondrial transcription regulatory element(s) that are functional in yeast mitochondria because the mouse mitochondrial promoters are not functional in yeast,

- the plasmid does not only encode the heterologous RNA of interest but also all the RNAs from the 17 kb mouse mitochondrial genome.
- the plasmid does not contain a synthetic mitochondrial transcription unit in which the isolated DNA encoding said heterologous RNA of interest is inserted between a promoter and a terminator that are functional in yeast mitochondria but the full unaltered mouse mitochondrial DNA genome (title of example 14, page 9; paragraphs [0065], [0066], [0067]; figure 5).

The transmitochondrial rho+ yeast contains mammalian mitochondrial transcription genes (POLRMT, TFB2M and TFAM) in the nuclear DNA which are absolutely necessary for the transcription of mammalian mtDNA in yeast (paragraphs [0019], [0068], [0076] and figure 6). These transgenes are absent in the nuclear DNA of the synthetic rho$^-$ yeast of the invention.

Furthermore, U.S. 2009/0098653 does not disclose or suggest:

- the step of isolating the mitochondria from said synthetic rho$^-$ yeasts (step 4 of the claimed method), and
- the step of extracting and purifying the heterologous RNA of interest from said mitochondria (step 5 of the claimed method).

The Examiner considers (page 5 of the present Office Action) that:

> The ordinary skilled artisan desiring to produce RNA in yeast mitochondria would have been motivated to combine the teaching of Bonnefoy 2001 teaching transformation of S. cerevisiae mitochondria with the teaching of US 2009/0098653 teaching transforming mitochondria with a plasmid comprising a gene which is expressed to produce RNA because Bonnefoy 2001 state that genetic manipulation of S. cerevisiae mitochondria are amenable to in vivo experimental analysis and should provide a useful model for other systems.

However, this is not true. First of all the problem of the invention is not to produce RNA in yeast mitochondria. This formulation of the problem of the invention is not correct

since it includes a part of its solution. As mentioned already, the problem of the invention is to specifically produce a heterologous RNA of interest *in vivo*.

As previously discussed on pages 10 and 11 of the response to the first Office Action, and in pages 14-15 of the response to the second Office Action, a reference should always be considered for everything it would have fairly taught a person having ordinary skill in the art. The ordinary skilled artisan is a specialist in recombinant DNA technology and gene expression in prokaryotic and eukaryotic expression systems.

Bonnefoy 2001 teach transforming *S. cerevisiae* mitochondria with a plasmid comprising a mitochondrial reporter gene (*ARG8*^{m}) which is expressed as a mitochondrial reporter protein (Arg8p) in cells from the recombinant rho^{+} yeasts having inserted the reporter gene in the mtDNA (page 98, 3rd and 4th paragraphs; page 99, 1st paragraph).

However, as previously mentioned, Bonnefoy 2001 teaches only how the mitochondrial genome can be manipulated using genetic transformation procedures. The concluding remarks suggest that the methods developed for manipulating *S. cerevisiae* mitochondrial genome would be usable for other single-cells eukaryote species.

As agreed by the Examiner in the two previous Office Actions and the instant Office Action (page 5 and page 17), Bonnefoy 2001 does not teach production and isolation of RNA.

On page 5 of the last OA, the Examiner considers that:

> It would have been obvious to one of ordinary skill in the art to use mitochondria to produce RNA because US 2009/0098653 teach that transforming yeast mitochondria with exogenous DNA allows for studying of transcription and production of mRNA in the mitochondria. The methods also teach means for RT-PCR, which allows for production of RNA in mitochondria.

This assertion is respectfully traversed for the reasons given on pages 10 and 11 of the response to the first OA and in pages 14-15 of the response to the second OA, a reference

should always be considered for everything it would have fairly taught a person having ordinary skill in the art.

U.S. 2009/0098653 teach transforming yeast mitochondria with a complete and unaltered exogenous (mammalian) mitochondrial genome and adding the appropriate mammalian genes for nuclear encoded mitochondrial proteins to these yeast strains to create a new *in vivo* mammalian mitochondrial model system which recapitulate species-specific mitochondrial processes as transcription and replication in these transmitochondrial yeast strains (paragraph [0019]). Therefore, U.S. 2009/0098653 teaches only transforming yeast mitochondria with a full mammalian mtDNA to study mitochondrial transcription.

U.S. 2009/0098653 does not teach transforming yeast mitochondria only with an exogenous DNA which is not related to mtDNA, *i.e.*, without a complete and viable mtDNA that can be replicated and is transcriptionally active (paragraph [0033]).

U.S. 2009/0098653 does not teach studying of transcription and production of any mRNA which is not encoded by mtDNA in mitochondria which do not contain a complete and viable mtDNA that can be replicated and is transcriptionally active (paragraph [0033]).

U.S. 2009/0098653 teaches means for RT-PCR which allows detecting the synthesis of a specific transcript in total cellular or mitochondrial RNAs. The RT-PCR uses a RNA template to generate a double-stranded DNA having a strand complementary to the RNA. Therefore RT-PCR does not allow for production of RNA but of DNA. RT-PCR is an in *vitro assay*. Therefore, RT-PCR does not allow for the production of DNA in mitochondria.

Therefore, the ordinary skilled artisan desiring to produce a heterologous RNA of interest would not have derived any motivation to combine the teachings of Bonnefoy 2001 and U.S. 2009/0098653 because these documents do not suggest a method for specifically producing a heterologous RNA in yeast.

As mentioned on page 2, line 19 to page 3, line 12 of the present specification and confirmed by the prior art cited by the Examiner (see for example, Fincham, Microbiol. Rev., 1989, 53, pages 165-166), the prior teaches cellular systems for specifically producing proteins of interest *in vivo* but not any cellular system for specifically producing RNAs of interest *in vivo*.

U.S. 2009/0098653 teaches that the transmitochondrial rho$^+$yeast system is a new *in vivo* mammalian mitochondrial system in a yeast cell (paragraph [0019]). This system is useful for studying mitochondria-originating human diseases (paragraphs [0039] and [0040]) and for producing amino acids/and or vitamins that are not naturally produced by yeasts (paragraph [0041]). Therefore, given the teaching of the prior art and the level of ordinary skill in the relevant art, one of ordinary skill in the art would have had no clue of potential utility of yeast mitochondria to specifically produce a heterologous RNA of interest.

Assuming *arguendo* that the routiner in the art would have transposed the transmitochondrial rho$^+$yeast system described in U.S. 2009/0098653 to the production of RNAs of interest, he or she would have no reasonable expectation of success for specifically producing a heterologous RNA of interest using the claimed method.

The ordinary artisan would have made transmitochondrial rho$^+$ yeast having inserted the DNA encoding the heterologous RNA of interest in the exogenous (mammalian) mitochondrial DNA and having further inserted mammalian mitochondrial transcription genes (POLRMT, TFB2M and TFAM) in the yeast nuclear DNA since U.S. 2009/0098653 teaches that mammalian mitochondrial transcription genes are absolutely necessary for the transcription of mammalian mtDNA in yeast (paragraphs [0019] and [0076]). The routiner in the art would have grown the transmitochondrial rho$^+$ yeasts, isolated the mitochondria and extracted the RNAs from the isolated mitochondria and thus would have arrived at a method

that is different from the claimed method that uses synthetic rho$^-$ yeasts to specifically produce a heterologous RNA of interest.

In addition, the ordinary artisan would have obtained a mixture of exogenous (mammalian) mitochondrial RNAs and heterologous RNA of interest. Therefore, the ordinary artisan could not produce the RNA interest in large amounts, for a low cost, and in a form which is stable and can be readily isolated insofar as the only RNAs which are produced in the mitochondria of the synthetic rho$^-$ yeasts are the RNA of interest alone or with the RNA of the mitochondrial transformation reporter gene (as mentioned above in paragraph 1).

In addition, the prior art teaches only that mitochondrial genes (yeast *COX2*, mouse *HSP*) or yeast nuclear genes (*ARG8*^{m}) encoding proteins which are normally imported into to the mitochondria (Arg8p) can be expressed in yeast mitochondria. Therefore, it was not obvious that any DNA of interest (not related to mitochondria) could be expressed in mitochondria.

Furthermore, the prior art teaches only that mitochondrial transcription occurs in the mitochondria of rho$^+$ cells (yeast or mammalian cells comprising an intact and functional mitochondrial DNA or mitochondrial DNA carrying local alteration in its sequence which inactivate the respiratory function of mitochondria (rho$^+$ mit$^-$)), *in organello* (*in vitro*; examples 8 and 13 of U.S. 2009/0098653) and *in vivo* (example 15 of US 2009/0098653). Therefore, it was not obvious that transcription of any DNA sequence of interest (not related to mitochondria) could occur efficiently *in vivo* the mitochondria of synthetic rho$^-$ yeast which lack all the mitochondrial DNA except the DNA encoding the mitochondrial transformation reporter gene.

It was also not obvious that the heterologous RNA produced in the mitochondria of such synthetic rho$^-$ yeast would be stable. Therefore, the above arguments rebut what is stated by the Examiner in page 6 of the present OA, given the teaching of the prior art and the level

of the ordinary skilled artisan at the time of the applicant's invention, said artisan would have had no reasonable expectation of success in practicing the claimed invention. For these reasons, the withdrawal of this rejection is respectfully requested.

Rejection—35 U.S.C. §103

Claim 4 was rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2001 and U.S. 2009/0098653 as applied to claims 1, 2, 5, 7, 8, 11 and 21-23, and further in view of Komiya, *et al.*, JBC, 1964, 269, 30893-3087 and Hwang, *et al.*, J. Virol., 2000, 74, 4074-4084. This rejection is not sustainable over the combination of Bonnefoy 2001 and U.S. 2009/0098653 for the reasons discussed above.

Furthermore, these secondary references do not suggest the elements missing from Bonnefoy 2001 and U.S. 2009/0098653 such as specifically producing a heterologous RNA in yeast mitochondria lacking mitochondrial DNA transformed with a mitochondrial transcription vector that is functional in yeast mitochondria, for the reasons discussed already in the response to the previous OA and acknowledged by the Examiner for the reasons mentioned above. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103

Claim 6 was rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2001 and U.S. 2009/0098653 as applied to claims 1, 2, 5, 7, 8, 11 and 21-23, and further in view of Anziano *et al.*, Proc. Natl. Acad. Sci. USA, 1991, 88, 5592-5596.

U.S. 2009/0098653 is not prior art for the subject matter of claim 6 for the reasons explained above. Support for claim 6 is found at the bottom of page 7 of the English translation of the priority document, see lines 30-33.

This rejection is not sustainable over Bonnefoy 2001 for the reasons discussed above in paragraph 3.2. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103

Claims 9, 10 and 12 were rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2001 and U.S. 2009/0098653 as applied to claims 1, 2, 5, 7, 8, 11 and 21-23, and further in view of Fincham, Microbiol. Rev., 1989, 53, 148-170

U.S. 2009/0098653 is not prior art for the subject matter of claims 9, 10 and 12 for the reasons explained above. The subject matter in these claims finds descriptive support respectively at page 10, line 17, page 10, line 20 and on page 13. This rejection is not sustainable over Bonnefoy 2001 for the reasons discussed above. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103

Claim 13 was rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2001 and U.S. 2009/0098653 as applied to claims 1, 2, 5, 7, 8, 11 and 21-23, and further in view of Kim *et al.*, Cancer Res., 1997, 57, 3115-3120.

U.S. 2009/0098653 is not prior art for the subject matter of claim 13 for the reasons explained above. Claim 13 finds descriptive support at the top of page 15 of the English translation of this application's priority document. This rejection is not sustainable over Bonnefoy 2001 for the reasons discussed above. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103

Claim 14 was rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2001 and U.S. 2009/0098653, as applied to claims 1, 2, 5, 7, 8, 11 and 21-23, and further in view of Dziembowski *et al.* and di Rago *et al.*, JBC, 1988, 263, 12564-12570.

U.S. 2009/0098653 is not prior art for the subject matter of claim 14 for the reasons explained above. Claim 14 finds descriptive support in the English translation of the priority document in the bottom half of page 15. This rejection is not sustainable over Bonnefoy 2001 for the reasons discussed above. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103

Claim 3 was rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2000 as applied to claims 1, 2, 5, 8-10 and 21-23, and further in view of Dziembowski *et al.*, JBC, 2003, 278, 1603-1611. This rejection is not sustainable over Bonnefoy 2000 for the reasons discussed above.

Furthermore, Dziembowski does not suggest the elements missing from the primary reference, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA as discussed already in the response to the previous OA and acknowledged by the Examiner for the reasons mentioned above. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103

Claim 4 was rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2000 as applied to claims 1, 2, 5, 8-10 and 21-23, and further in view of Komiya, *et al.*, JBC, 1964, 269, 30893-3087 and Hwang, *et al.*, J. Virol., 2000, 74, 4074-4084. This rejection is not sustainable over Bonnefoy 2000 for the reasons discussed above.

Furthermore, these secondary references do not suggest the elements missing from Bonnefoy 2000, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA as discussed already in the response to the previous Office Action and acknowledged by the Examiner for the reasons mentioned above. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103

Claim 6 was rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2000 as applied to claims 1, 2, 5, 8-10 and 21-23, and further in view of Anziano, *et al.*, Proc. Natl. Acad. Sci. USA, 1991, 88, 5592-5596. This rejection is not sustainable over Bonnefoy 2000 for the reasons discussed above.

Furthermore, Anziano does not suggest the elements missing from the primary reference, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA as discussed already in the response to the previous OA and acknowledged by the Examiner for the reasons mentioned above. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103

Claim 13 was rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2000 as applied to claims 1, 2, 5, 8-10 and 21-23, and further in view of Kim, *et al.*, Cancer Res., 1997, 57, 3115-3120. This rejection is not sustainable over Bonnefoy 2000 for the reasons discussed above. Furthermore, Kim does not suggest the elements missing from the primary reference, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA as discussed already in the response to the previous OA and acknowledged by the Examiner for the reasons mentioned above. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103

Claim 14 was rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2000, as applied to claims 1, 2, 5, 8-10 and 21-23, and further in view of Dziembowski *et al.* and di Rago *et al.*, JBC, 1988, 263, 12564-12570. This rejection is not sustainable over

Bonnefoy 2000 for the reasons discussed above. Furthermore, di Rago and Dziembowski do not suggest the elements missing from the primary reference, such as expression of a heterologous RNA in yeast mitochondria lacking mitochondrial DNA as discussed already in the response to the previous OA and acknowledged by the Examiner for the reasons mentioned above. Accordingly, this rejection cannot be sustained.

Rejection—35 U.S.C. §103

Claims 1, 2, 5, 8-11 and 21-23 were rejected under 35 U.S.C. 103(a) as being unpatentable over Bonnefoy 2001, in view of Bonnefoy 2000. This rejection cannot be sustained because the cited prior art does not disclose all the elements of the claimed method, suggest the combination of elements of the claimed method, or provide a reasonable expectation of success for producing a heterologous RNA of interest using this method.

The Examiner considers (page 17 of the present OA) that:

> The skilled artisan desiring to RNA in yeast mitochondria would find some motivation to combine the teaching of Bonnefoy 2001 teaching transformation of S. cerevisiae with the teaching of Bonnefoy 2000 teaching transforming mitochondria with a plasmid comprising a gene which is expressed to produce RNA because Bonnefoy 2001 state that genetic manipulation of S. cerevisiae mitochondria are amenable to in vivo experimental analysis and should provide a useful model for other system.

The Applicants respectfully traverse this assertion. First of all, the problem of the invention is not to produce RNA in yeast mitochondria. The Examiner's formulation of the problem of the invention is not correct since it includes a part of its solution. As mentioned already, the problem of the invention is to specifically produce a heterologous RNA of interest *in vivo*.

As previously discussed at pages 10 and 11 of the response to the first OA and in pages 14-15 of the response to the second OA, a reference should always be considered for everything it would have fairly taught a person having ordinary skill in the art. The ordinary

skilled artisan is a specialist in recombinant DNA technology and gene expression in prokaryotic and eukaryotic expression systems.

Bonnefoy 2000 and Bonnefoy 2001 both teach transforming *S. cerevisiae* mitochondria with a plasmid comprising a mitochondrial reporter gene (*ARG8*^{m}) which is expressed as a mitochondrial reporter protein (Arg8p) in cells from the recombinant rho^{+} yeasts having inserted the reporter gene in the mtDNA (Bonnefoy 2000, page 1037, 1st column, beginning of 3rd paragraph; Bonnefoy, 2001, page 98, 3rd and 4th paragraphs; page 99, 1st paragraph).

As discussed in the response to the previous Office Actions, Bonnefoy 2001 teaches only how the mitochondrial genome can be manipulated using genetic transformation procedures. The concluding remarks suggest that the methods developed for manipulating *S. cerevisiae* mitochondrial genome would be usable for other single-cells eukaryote species.

As agreed by the Examiner in the two previous Office Actions and the last OA at page 5 and page 17, Bonnefoy 2001 does not teach production and isolation of RNA. The Examiner considers on pages 17-18 of the present OA that:

> It would have been obvious to one of ordinary skill in the art to use mitochondria to produce RNA because Bonnefoy 2000 teach that translation initiation in mitochondrial systems is poorly understood, and that examination of mRNA structure and genetic analyses makes it possible to ascertain features of translation initiation in fungal mitochondria.

The Applicants respectfully traverse this assertion. As discussed on pages 10 and 11 of the response to the first OA and in pages 14-15 of the response to the second OA, a reference should always be considered for everything it would have fairly taught a person having ordinary skill in the art.

Bonnefoy 2000 pertains to the examination of translation initiation, *i.e.*, protein synthesis in the mitochondria of recombinant rho^{+} yeasts (page 1037, 1st column, last paragraph; page 1040, first column , 2nd paragraph; figures 1B and 3; *Discussion*, page 1042

to page 1043, 1st column, line 4). In addition, examination of mRNA structure and genetic analyses do not refer to heterologous RNA production. As mentioned above, Bonnefoy 2000 does not teach producing RNA. Bonnefoy 2000 teaches producing a reporter protein (Arg8p).

Bonnefoy 2000 would have motivated one of ordinary skill in the art to make other yeast mitochondrial recombinants to search for *cis*- or *trans*-acting elements of the mitochondrial translation system that govern start site selection in yeast mitochondria (see page 1037, 2nd column, 1st paragraph; page 1044, 1st column, end of discussion). Therefore, the ordinary skilled artisan desiring to produce a heterologous RNA of interest would have found no motivation to combine the teaching of Bonnefoy 2000 and Bonnefoy 2001 because these documents do not suggest a method for specifically producing a heterologous RNA in yeast.

Assuming *arguendo* that the skilled artisan would have combined the teaching of Bonnefoy 2000 with the teaching of Bonnefoy 2001, he would have no reasonable expectation of success for producing a heterologous RNA of interest using the claimed method because Bonnefoy 2000 and Bonnefoy 2001 neither disclose or suggest producing a heterologous RNA, isolating yeast mitochondria or extracting and purifying RNA from yeast mitochondria. Furthermore, Bonnefoy 2000 and Bonnefoy 2001 neither suggest a process of expressing heterologous RNA in mitochondria lacking mitochondrial DNA (synthetic rho$^-$ yeast).

As mentioned in the present application at page 2, line 19 to page 3, line 12 and confirmed by the prior art cited by the Examiner (see for example, Fincham, Microbiol. Rev., 1989, 53, pages 165-166) the prior teaches cellular systems for specifically producing proteins of interest *in vivo* but not any cellular system for specifically producing RNAs of interest *in vivo*.

Given the teaching of the prior art and the level of ordinary skill in the relevant art, the ordinarily skilled artisan would have had no clue of the potential utility of yeast mitochondria to specifically produce a heterologous RNA of interest.

Even if one of ordinary skill in the art had transposed the system for producing proteins described in Bonnefoy 2000 and Bonnefoy 2001 to the production of RNAs of interest, s/he would have made recombinant rho+ yeasts having inserted the DNA encoding the heterologous RNA of interest in their mt-DNA, grown the recombinants, isolated the mitochondria and extracted the RNA from the isolated mitochondria, thus arriving at a method that is different from the claimed method that uses synthetic rho^- yeasts to produce a heterologous RNA of interest.

In addition, the ordinary artisan would have obtained a mixture of endogenous yeast mt-RNAs and heterologous RNA of interest and would not have produced the RNA interest in large amounts, for a low cost, and in a form which is stable and can be readily isolated insofar as the only RNAs which are produced in the mitochondria of the synthetic rho^- yeasts are the RNA of interest alone or with the RNA of the mitochondrial transformation reporter gene (as mentioned above).

In addition, the prior art teaches only that mitochondrial genes (*COX2*) or yeast nuclear genes ($ARG8^m$) encoding proteins which are normally imported into to the mitochondria (Arg8p) can be expressed in yeast mitochondria. Therefore, it was not obvious that any DNA of interest (not related to mitochondria) could be expressed in mitochondria.

Furthermore, the prior art teaches only that transcription occurs in the mitochondria of rho^+ yeast, *i.e.*, yeasts comprising an intact and functional mitochondrial DNA or mitochondrial DNA carrying local alteration in its sequence which inactivate the respiratory function of mitochondria (rho^+ mit^- yeast; page 5, lines 31-36 of the present application).

Therefore, it was not obvious that transcription of any DNA sequence of interest (not related to mitochondria) could occur efficiently and that the heterologous RNA produced could be stable in the mitochondria of synthetic rho$^-$ yeast which lack all the mitochondrial DNA except the DNA encoding the mitochondrial transformation reporter gene. Therefore, the above arguments rebut what is stated by the Examiner in page 18 of the present OA, given the teaching of the prior art and the level of the ordinary skilled artisan at the time of the applicant's invention, said skilled artisan would have had no reasonable expectation of success in practicing the claimed invention. Consequently, this rejection cannot be sustained.

Conclusion

In view of the amendments and remarks above, the Applicants respectfully submit that this application is now in condition for allowance. An early notice to that effect is earnestly solicited.

Respectfully submitted,

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